RSC Advances

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/advances

Determination of sparfloxacin and besifloxacin hydrochlorides using gold nanoparticles modified carbon paste electrode in micellar medium

Ali K. Attia[a,*], Amr M. Badawy[b], Samr G. Abd-Elhamid[b]

[a]*National Organization for Drug Control and Research, P.O. Box 29, Cairo, Egypt.*

[b]*Faculty of Pharmaceutical Sciences & Pharmaceutical Industries, University of Future, Cairo, Egypt.*

Abstract

A gold nanoparticles modified carbon paste electrode (AuCPE) was used to study the electrochemical behavior of sparfloxacin HCl (SPAR) and besifloxacin HCl (BESI) using cyclic and differential pulse voltammetry modes in presence of micellar medium. Effect of different surfactants on peak current was studied in Britton-Robinson buffer solution of pH 2. Sodium dodecyl sulphate is the optimum surfactant based on the enhancement of the peak current. The modified electrode shows a highly sensitive sensing that giving an excellent response for SPAR and BESI. The peak current varied linearly over the concentration ranges from 1.1 x 10^{-7} mol L^{-1} to 3.3 x 10^{-6} mol L^{-1} and from 2.2 x 10^{-6} mol L^{-1} to 5.5 x 10^{-5} mol L^{-1} with determination coefficients of 0.9976 and 0.9984 in case of SPAR and BESI, respectively. The recoveries and the relative standard deviations were found in the following ranges: 99.97-101.4% and 0.63-1.48% for SPAR and 99.89-101.1% and 0.85-1.76% for BESI. The detections limits were 2.87 x 10^{-8} and 3.76 x 10^{-7} mol L^{-1} for SPAR and BESI, respectively. The proposed method has been successfully applied to determine SPAR and BESI in biological fluids.

Keywords: Gold nanoparticles, Sparfloxacin HCl, Besifloxacin HCl, Biological fluids.

*Corresponding author. Tel.: 002 0235851278; Fax: 002 0235855582

E-mail: alikamal1978@hotmail.com (Ali K. Attia*).

1. Introduction

SPAR is a third generation fluoroquinolones, used in the treatment of lung infection, urinary tract infection and cutaneous allergy. SPAR is receiving attention due to its broad spectrum activity, potency and excellent pharmacokinetic profiles.[1,2] BESI is a fluoroquinolone antibacterial with activity against gram-positive and gram-negative bacteria due to the inhibition of both bacterial DNA gyrase and topoisomerase IV.[3,4]

Electrochemical methods,[5-9] chromatographic methods,[10-16] spectrophotometric methods,[17-24] chemiluminescence method,[25] and conductometric method,[26] have been reported for quantitative determination of SPAR in bulk and pharmaceutical dosage forms. Other chromatographic methods,[27-30] and spectrophotometric methods,[31,32] have been reported for quantitative determination of BESI in bulk and pharmaceutical dosage forms.

Gold nanoparticles (GNPs) are very important in electrochemistry based on the formation of monolayers on the electrode surface leading to large surface area, good and high conductivity, so they are used in electrochemical studies.[33-37] There is no attempt to study the electrochemical behavior of BESI. The reported electrochemical methods used to determine SPAR using glassy carbon electrode,[5-7] polagraphic method using dropping mercury electrode,[8] and carbon paste electrode (CPE).[9]

The need to determine drugs at high sensitivity and wider ranges than the reported methods, therefore in the present study, it was intend to develop a rapid, economical, simple, precise and more sensitive voltammetric method for the determination of

SPAR and BESI in bulk and dosage forms and biological fluids using cyclic voltammetry (CV) and differential pulse voltammetry (DPV) techniques showing very low detection limits and wider linear ranges for the used drugs.

2. Experimental

2.1. Materials and reagents

SPAR was provided from Pharaonia pharmaceuticals, Cairo, Egypt, BESI was purchased from Bausch & Lomb Inc., New York, USA. Spara tablets were provided from Global Napi Pharmaceuticals, Cairo, Egypt. Each tablet is labeled to contain 200 mg of SPAR. Besivance eye drops were provided from Bausch & Lomb Inc., New York, USA. It is labeled to contain 0.6% per 5 mL of BESI.

All chemicals and reagents used throughout the work were of analytical reagent grade and solutions were made with doubly distilled water. Stock solutions of 1.0 x 10^{-3} mol L^{-1} of SPAR and BESI were prepared by dissolving the accurately weighed amount in doubly distilled water and methanol, respectively. The stock solutions were stored in dark bottle and kept in the refrigerator for no more than seven days. Ascorbic acid (AA), uric acid (UA), sodium dodecyl sulphate (SDS), tween 80 and cetyltrimethyl ammonium bromide (CTAB) were provided from Sigma-Aldrich, Taufkirchen, Germany. Britton-Robinson (BR) buffer solutions (pH 2-9) were used as supporting electrolytes. BR buffers were made by mixing a solution of 0.04 mol L^{-1} phosphoric acid, 0.04 mol L^{-1} acetic acid and 0.04 mol L^{-1} boric acid which obtained from El-Nasr pharmaceutical company, Cairo, Egypt. Buffer solutions were adjusted by adding the necessary amount of 2.0 mol L^{-1}NaOH solutions in order to obtain the appropriate pH. Graphite powder and mineral oil were supplied from Sigma-Aldrich, Taufkirchen, Germany.

2.2. Electrochemical measurements

All voltammetric measurements were performed using a pc-controlled AEW2 electrochemistry work station and data were analyzed with ECprog3 electrochemistry software, manufactured by SYCOPEL SCIENTIFIC LIMITED (Tyne & Wear, UK). The one compartment cell with the three electrodes was connected to the electrochemical workstation through a C3-stand. Platinum wire from was employed as auxiliary electrode. All the cell potentials were measured with respect to Ag/AgCl (3 mol L^{-1} NaCl) reference electrode from. Glass cell (5 mL) was used for electrochemical measurements. All electrodes and the C3 stand were obtained from BASi (Indiana, USA). A JENWAY 3510 pH meter (Staffordshire, England) with glass combination electrode was used for pH measurements. All the electrochemical experiments were performed at an ambient temperature of 25 ˚C.

2.3. Gold nanoparticles modified electrode

CPE with a diameter of 3 mm was prepared as described before,[38] and then it was immersed into a 6 mmol L^{-1} hydrogen tetrachloroaurate $HAuCl_4$ solution containing 0.1 mol L^{-1} KNO_3 prepared in doubly distilled water and deaerated by bubbling with nitrogen. A constant potential of -0.4 V vs. Ag/AgCl was applied for 400 s.[39] Then, the modified electrode AuCPE was washed with doubly distilled water and dried carefully by a paper without touching the surface and then left to dry in air for 10 min before being used.

2.4. Effect of surfactants

The cyclic voltammogramsof 1 x 10^{-3} mol L^{-1} SPAR and BESI (in BR buffer, pH 2) were studied on AuCPE upon successive additions of each of the following surfactants: (SDS), (tween 80) and (CTAB) of the same concentration of 1 x 10^{-2} mol L^{-1}) to obtain the optimum one.

2.5. Determination of SPAR and BESI in bulk

Aliquots of SPAR and BESI solutions (1 x 10^{-3} mol L^{-1}) were added to the electrolytic cell containing 5 ml of BR buffer of pH 2. The solution was stirred for 5 s at open circuit conditions in presence of at AuCPE/SDS and voltammetric analyses were carried out and the voltammograms were recorded at scan rate = 10 mV s^{-1}, pulse width = 25 ms and pulse amplitude = 50 mV.

2.6. Determination of investigated drugs in pharmaceutical preparations

In two separate 100 mL calibrated flasks add an amount of Spara tablets and Besivance eye drops equivalent to prepare 1 x 10^{-3} mol L^{-1} SPAR and BESI solutions, and then add 70 mL of methanol. The content of the flask was sonicated for about 15 min and then made up to the volume with the same solvent. The solution was filtered to separate the insoluble excipients. Aliquots of the drugs solution were introduced into the electrolytic cell and the general procedure was carried out.

2.7. Analysis of drugs in urine and plasma

Urine and plasma samples were obtained from healthy volunteers; standard SPAR was dissolved in urine to make 1 x 10^{-3} mol L^{-1}concentration. Successive additions of SPAR (1 x 10^{-3} mol L^{-1}) were added to the voltammetric cell containing 5 mL BR buffer of pH 2. Different volumes of BESI 1 x 10^{-3} mol L^{-1} (0.2 - 1 mL) were added to different tubes containing 1 mL of plasma and 3 mL of acetonitrile shake very well then centrifuge, then take 1 mL of the supernatant and complete the volume to 10 mL with methanol, finally take 0.5 mL of the successive additions of BESI 1 x 10^{-3} mol L^{-1} were added to 5 mL BR buffer pH 2. All experiments were performed in compliance with the relevant laws and institutional guidelines, and the institutional committees (NODCAR, Egypt) have approved these experiments and any

experimentation with human subjects. Informed consent was obtained from all participants or their relatives.

3. Results and discussion

3.1. Optimization of the method

The proposed voltammetric method used for the determination of SPAR and BESI is safe because it does not use toxic chemicals.

3.1.1. Electrochemical behavior of SPAR and BESI

Figures 1 and 2 show the cyclic voltammograms of 1 x 10^{-3} mol L^{-1} SPAR and BESI solutions, in BR buffer of different pH values ranging from 2 to 9 at CPE, exhibit anodic peaks with no peaks on the reverse scan, suggesting the irreversible nature of the electrode reaction. From the figures we note that the anodic peak potential has shifted negatively with the increase of the solution pH indicating that the oxidation of SPAR and BESI is pH dependent reaction and that protons have taken part in their electrode reaction processes. The oxidation of SPAR occurs through the loss of two electrons and two protons one attached to nitrogen atom in the piperazine ring and the other proton from water molecule. For BESI, the oxidation process occurs by the loss one electron from nitrogen atom and one proton from its adjacent carbon atom in azepan ring. The peak potential for SPAR and BESI oxidation varies linearly with pH (over the entire pH range) which fit to the linear regression equations of E (V) = 0.0699 - 0.0582 pH, with determination coefficient (r^2) = 0.9952, and E (V) = 0.0476 - 0.0593 pH, r^2 = 0.9936, in case of SPAR and BESI, respectively. The highest oxidation peak current of SPAR and BESI were obtained at buffer of pH 2. The slopes were found to be -58.2 and -59.3 mV/pH units, which is close to the theoretical value of -59 mV. This indicated that the number of protons and transferred electrons

involved in this mechanism is equal.[40] The results show that BR buffer of pH 2 was chosen as the optimum pH used in subsequent experiments.

Fig. 1

Fig. 2

3.1.2 Effect of different surfactants

Different successive additions of different surfactants, SDS, tween 80 and CTAB of the same concentration (1 x 10^{-2} mol L^{-1}) were added to the voltammetric cell containing 1 x 10^{-3} mol L^{-1} of SPAR and BESI in BR buffer of pH 2 and the cyclic voltammograms were recorded at CPE as shown in Fig. 3. The maximum current values (15.92 µA, 13.4 µA and 12.16 µA) of SPAR was found in the presence of 8 x 10^{-5}, 2 x 10^{-5} and 4 x 10^{-5} mol $L^{-1}$of SDS, tween 80 and CTAB, respectively, while The maximum current values (48.0 µA, 19.4 µA and 34.4 µA) in case of BESI was found at 8 x 10^{-5}, 1 x 10^{-4} and 8 x 10^{-5} mol L^{-1} of SDS, tween 80 and CTAB, respectively.

SPAR and BESI are positively charged and attracted to the negative charges of the head of SDS surfactant, therefore, 8 x 10^{-5} mol L^{-1} of SDS was chosen as the optimum surfactant in this study. Since SPAR and BESI are positively charged in solution, SDS is the most suitable surfactant as anionic surfactant for the determination of theses drugs in micellar medium.

Fig. 3

3.1.3. Effect of gold nanoparticles

Figure 4 shows the cyclic voltammograms of 1 x 10^{-3} mol L^{-1} of SPAR and BESI in BR buffer pH 2 at scan rate of 100 mVs^{-1} at CPE and AuCPE in absence and in presence of SDS (8 x 10^{-5} mol L^{-1}). At CPE electrode, the oxidation peak was observed at 0.98 V and 1.26 V in case of SPAR and BESI, respectively, with current

response 8.86 μA, and 19.98 μA in case of SPAR and BESI, respectively, whereas at CPE/SDS, the current response increases to 15.92 μA and 48.0 μA in case of SPAR and BESI, respectively, due to the aggregation of surfactants on CPE surface in the form of bilayers, cylinders, or surface micelles could explain the increase in current in the presence of surfactants.[41] At AuCPE the current response increases to 21.95 μA and 53.22 μA in case of SPAR and BESI, respectively, due to the enhancement of the electron transfer process and a larger intrinsic surface area of the modified electrode. Whereas, AuCPE/SDS the current response increase to 29.17 μA and 76.65 μA in case of SPAR and BESI, respectively, due to the aggregation of surfactants on the electrode surface after the electro-deposition of Au particles on CPE surface resulted in an observable increase in the peak current, which indicated a decrease in the potential of oxidationfrom 0.98 to 0.92 V for SPAR and from 1.27 to 1.19 V in case of BESI. The results confirmed the key role played by surfactant and Au nanoparticles on the catalytic oxidation which enhances the electrochemical reaction.[42]

Fig. 4

3.1.4. Effect of the scan rate

The influence of scan rate (ν ranging from 25 to 250 mV s^{-1}) on the oxidation peak currents of SPAR and BESI (1 x 10^{-3} mol L^{-1}) was studied at AuCPE/SDS in BR buffer (pH 2) and a linear relationship is found for the logarithm of the oxidation peak currents and the logarithm of the scan rates (Fig. 5). For SPAR, The oxidation peak current increases linearly with the linear regression equation as log I = -0.013 + 0.59 log ν, r^2 = 0.9982, and for BESI. The oxidation peak current increases linearly with the linear regression equations as log I = 0.106 + 0.81 log ν, r^2 = 0.9920. The slopes 0.59 (SPAR) and 0.81 (BESI) suggest that the oxidation reactions at the electrode surface take place under adsorption-diffusion controlled process.[43]

Fig. 5

In case of irreversible electrode process, the peak potential (E_p) and scan rate (υ) are defined by the following Laviron equation.[44]

$$E_p = E^o + 2.303\ RT/\alpha nF\ [\log RTK^o/\alpha nF + \log \upsilon]$$

where α is the electron transfer coefficient, n is the number of electrons, T is the temperature (298 K), R is the gas constant (8.314 J K mol^{-1}) and F the Faraday constant (96485 C mol^{-1}), respectively. Thus we can calculate αn from the slope of the relation between Ep versus log υ

In this case, the slope values are 0.055 and 0.092, for SPAR and BESI, respectively; αn values were calculated to be 1.075 and 0.642. Generally, α (electron transfer coefficient) was assumed to be 0.5. Thus, the value of electrons number n = 2.15 (≈2) and 1.286 (≈1) were obtained confirming the proposed electro-oxidation mechanisms of SPAR and BESI as shown in Fig. 6.

Fig. 6

3.1.5. Effect of accumulation time

The effect of accumulation time (t_{acc}) on the anodic peak current (I) of SPAR and BESI was studied at AuCPE/SDS. Sharp increasing in current value was obtained up to 10 s and 5 s for SPAR and BESI, respectively, and then the current practically decreased with increasing time. So accumulation time of 10 s and 5 s were chosen as the optimum accumulation time for SPAR and BESI, respectively (Fig. 7).

Fig. 7

3.2. Chronoamperometric studies

Chronoamperometry technique was used to obtain the diffusion coefficients of SPAR and BESI. Figs. 8A and 9A show the chronoamperograms of AuCPE/SDS in the presence of different concentrations of SPAR and BESI. The current varied linearly

with $t^{-1/2}$ (the minus square roots of time) as shown in Figs. 8B and 9B. Using the slopes of these lines, the diffusion coefficient of SPAR and BESI can be calculated using Cottrell's equation: $I = nFAC (D/\pi t)^{1/2}$ where n is the number of electrons involved in the electro-oxidation process (n = 2 and 1 for SPAR and BESI, respectively), F is the Faraday constant, C is the analyte concentration, D is the diffusion coefficient, and A is electrode area.[45] The slopes of the resulting straight lines were then plotted against SPAR and BESI concentrations (Figs. 8C and 9C). From the resulting slopes, the diffusion coefficients were found to 1.62 x 10^{-7} cm^2 s^{-1} and 4.81 x 10^{-7} cm^2 s^{-1} for SPAR and BESI, respectively.

Figs. 8 (D,E) and 9 (D,E) show the effect of SPAR and BESI concentrations on the response and the recovery times on the AuCPE/SDS electrode with respect to the shortest response time and the recovery time. The response time decreases and the recovery time increases with increasing SPAR and BESI concentrations, which may be attributed to the adsorption/desorption process of SPAR and BESI on the electrode surface. Finally, AuCPE/SDS electrode shows fast response and recovery times for SPAR and BESI in comparison with the reported methods which were not interested in studying the response and recovery times.

Fig. 8

Fig. 9

3.3. Method validation

3.3.1. Interference of SPAR and BESI with ascorbic acid and uric acid

The ability of sensor to discriminate between the interfering species commonly present in similar physiological environment and the target analyte is very important. Ascorbic acid (AA) is a naturally occurring organic compound with antioxidant properties. Humans require it as part of their nutrition.[46] Uric acid (UA) is the primary

end product of purine metabolism in the human body.[47] Extreme abnormalities of UA levels are symptomatic of several diseases, including gout, hyperuricemia and Lesch-Nyan disease.[48] Therefore, determination of SPAR and BESI in the presence of AA and UA is very important for the clinical point of view.

Differential pulse voltammetry (DPV) was used to determine SPAR and BESI (1 x 10^{-5} mol L^{-1}) in presence of equimolar solutions of AA and UA (1 x 10^{-4} mol L^{-1}), the applied scan rate was 10 mV s^{-1}. Fig. 10 shows the differential pulse voltammograms at CPE and AuCPE/SDS in BR buffer (pH 2). The anodic peak current increased from 10.42 μA (0.993 V) in case of CPE to 18.98 μA (0.962 V) in case of AuCPE/SDS, while in case of BESI, the anodic peak current increased from 24.3 μA (1.3 V) in case of CPE to 48.90 μA (1.2 V) in case of AuCPE/SDS.

The pKa values of AA and UA are 4.2 and pKa 5.4, respectively. Therefore, AA and UA exist as anions at pH higher than 4.2 and 5.4 whereas at pH less than 4.2 and 5.4, they appear as anionic and neutral species.[49-51] Therefore, the anionic and neutral species of AA and UA are unaffected with SDS micelles which are electrostatically attracted to the positively charged cations of SPAR and BESI through the negatively charged head of SDS showing an increase of peak currents and improve the sensitivity and selectively of the sensor towards SPAR and BESI in the presence of AA and UA showing catalytic effect.

Fig. 10

3.3.2. Determination of SPAR and BESI in the bulk

Fig. 11 shows the calibration plots of SPAR and BESI at AuCPE/SDS over the concentration ranges from 1.1 x 10^{-7} mol L^{-1} to 3.3 x 10^{-6} mol L^{-1}, r^2 = 0.9976 in case of SPAR and from 2.2 x 10^{-6} mol L^{-1} to 5.5 x 10^{-5} mol L^{-1}, r^2 = 0.9984 in case of BESI.

Validation of the proposed methods was assessed as per the International Conference on Harmonization (ICH) Tripartite Guideline Q2 (R1) was followed to validate the method.[52]

For SPAR, the limit of detection (LOD) and the limit of quantification (LOQ) were found to be 2.87 x 10^{-8} mol L^{-1} and 9.57 x 10^{-8} mol L^{-1}, respectively. In case of BESI, LOD and LOQ were found to be 3.76 x 10^{-7} mol L^{-1} and 1.25 x 10^{-6} mol L^{-1}, respectively. Both LOD and LOQ values confirm the sensitivity of AuCPE/SDS.

The recoveries were found in the following ranges: 99.97-101.4% for SPAR and 99.89-101.1%, for BESI. The relative standard deviations were found in the following ranges: 0.63-1.48% and 0.85-1.76% for SPAR and BESI, respectively. The results are shown in Table 1.

Fig. 11

Table 1

The repeatability of the proposed DPV procedure was investigated on five measurements of 2.5 x 10^{-7} mol L^{-1} SPAR solution and 3.25 x 10^{-6} mol L^{-1} BESI solution, the relative standard deviation (RSD) was found to be 1.23% (SPAR) and 1.57% (BESI) indicating good results.

Table 2

3.3.3. Assay of SPAR and BESI in their pharmaceutical preparations

Standard addition method was successfully applied to the direct determination of SPAR in Spara tablets and BESI in Besivance eye drops using AuCPE/SDS without the necessity for the sample pretreatment or time consuming extraction steps prior to the analysis. Based on the average of five replicate measurements, the values of mean recovery and mean RSD values for SPAR were 100.14% and 0.142%; respectively

the values of mean recovery and mean RSD for BESI were 100.17% and 0.234%, respectively. The obtained results in Table 3 were in acceptable limits.

Tabel 3

3.3.4. Assay of SPAR and BESI in urine and plasma

The proposed voltammetric method is used to determine SPAR and BESI in urine and plasma samples. The results gives linear range of 2.22 x 10^{-7} - 2.80 x 10^{-6} mol L^{-1}, r^2 = 0.9964, the LOD was 4.15 x 10^{-8} mol L^{-1} and LOQ was 1.38 x 10^{-7} mol L^{-1} in case of SPAR and shows linear range of 8 x 10^{-6} - 4.5 x 10^{-5} mol L^{-1}, r^2 = 0.9980, the LOD was 9.12 x 10^{-7} mol L^{-1} and LOQ was 3.04 x 10^{-6} mol L^{-1} for BESI (Table 1). Five different concentrations on the calibration curve are chosen to be repeated five times to evaluate the accuracy and precision of the proposed method which is represented in Table 4.

Table 4

4. Conclusion

In the present work, novel sensor based on modification of CPE with gold nanoparticles in presence of surfactants was used for electrochemical determination of SPAR and BESI. The advantages of the gold nanoparticles/surfactant enhanced the sensitivity of the CPE towards these drugs. The results showed that the method is easy-to-handle, rapid, ecofriendly, simple and sensitive for the determination of SPAR and BESI in human urine and plasma with good precision, accuracy, selectivity and very low detection limit. The high percentage of recoveries in pharmaceutical formulations without any treatment confirms the suitability of the proposed method. Further, due to stability, accuracy and low cost, the method offers promise as a substitute for the previous approaches used in routine analysis.

Acknowledgment

The authors would like to express their gratitude to National Organization for Drug Control and Research (NODCAR, Egypt) for providing instruments and chemicals.

References

1 K. Sato, Y. Inoue, T. Fujii, H. Aoyama, M. Inoue, *Antimicrob. Agent Chemother.*, 1986, **30**, 777-780.

2 P. B. Fernandes, *Quinolones: Proceedings of an International Telesymposium*. Prous Science Publisher: Bracelona, 1989.

3 M. E. Tepedino, W. H. Heller, D. W. Usner, L. S. Brunner, T. W. Morris, W. Haas, M. R. Paterno, T. L. Comstock, *Curr. Med. Res. Opinion*, 2009, **25**, 1159-1169.

4 S. C. Lal, S. Amit, K. Sokindra, D. K. Majumdar, *J. Drug Delivery Therap.*, 2014, **4**, 39-44.

5 K. G. Kumar, P. Augustine, R. Poduval, S. John, *Pharmazie*, 2006, **61**, 291-292.

6 A. Radi, T. Wahdan, Z. Anwar, H. Mostafa, *Electroanalysis*, 2010, **22**, 2665-2671.

7 A. Radi, T. Wahdan, Z. Anwar, H. Mostafa, *Drug Test. Anal.*, 2010, **2**, 397-400.

8 S. Jain, N. K. Jain, K. S. Pitre, *J. Pharm. Biomed.Anal*., 2002, **29**, 795-801.

9 M. A. EL-Ries, A. A. Wassel, N. T. Abdel-Ghani, M. A. El-Shall, *Anal. Sci.*, 2005, **12**, 1249-1254.

10 D. Kowalczuk, H.Hopkaa, R. Pietre, *Chem. Anal. (Warsaw)*, 2004, **49**, 201-211.

11 R. Singh, A. Pathak, P. Chawla, *Ind. J. Pharm. Biol. Res.*, 2013, **1**, 95-101.

12 G. Nirupa, U. M. Tripathi, *Int. J. Res. Pharm Biomed. Sci.*, 2013, **4**, 27-36.

13 K. B. Desai, M. A. Patel, P. K. Pradhan, S. Dey, D. U. M. Upadhyay, *Asian J. Pharm. Res. Develop.*, 2013, **1**, 55-62.

14 M. M. Sebaiy, A. A. El-Shanawany, S. M. El-Adl, L. M. Abdel-Aziz, H. A. Hashem, *Asian J. Pharm. Res.*, 2011, **1**, 119-125.

15 Y. L. Feng, *Anal. Lett.*, 2001, **34**, 2693-2700.

16 S. S.Panchumrathy, D. R. Garikapati, G. R. Padala, *J. Chem. Pharm. Sci.*, 2013, **6**, 120-133.

17 M. M. Sebaiy, A. A. El-Shanawany, S. M. El-Adl, L. M. Abdel-Aziz, *Asian J. Res. Pharm. Sci.*, 2011, **1**, 131-139.

18 A. Srikar, K. P. Channabasavaraj, G. Dharmamoorty, N. Valmiki, C. Chinnappa, T. V. Babu, *J. Pharm. Sci. Res.*, 2009, **2**, 13-15.

19 J. Shah, M. R. Jan, Inayatullah, S. Shah, *J. Mex. Chem. Soc.*, 2012, **56**, 109-114.

20 P. Kamlesh, R. Badmanaban, C. N. Patel, *J. Chem. Pharm. Res.*, 2010, **2**, 528-532.

21 S. S. Parimala, P. A. Jyothi, and K.Tejaswi, *Int. J. Innovative Pharm. Res.*, 2013, **24**, 306-309.

22 A. Srikar, D. Swapna, G. Swathi, I. Swapna, D. Sucharitha, *Int. J. Pharm. Tech.***,** 2010, **2**, 16-22.

23 M. S. Arayne, N. Sultana, S. N. Ali, *Med. Chem.*, 2013, **3**, 271-275.

24 N. Sreekanth, B. Z. Awen, C. B. Rao, *Res. J. Pharm. Bio. Chem. Sci.*, 2010, **1**, 9-13.

25 G. A. Saleh, H. F. Askal, I. H. Refaat, F. A. M. Abdel-aal, *Asian J. Biomed. Pharm. Sci.*, 2014, **4**, 39-49.

26 J. Al-Mustafa, A. Shinar, *Jordan J. Chem.*, 2013, **8**, 237-246.

27 M. C. Costa, A. T. Barden, J. M. Andrade, T. P. Oppe, E. E. *Talanta*, 2014, **119**, 367-374.

28 D. R. Arnold, C. P. Granvil, K. W. Ward, J. W. Proksch, *J.* Chromatogr. *B*, 2008, **867**, 105-110.

29 Z. Wang, S. Wang, F. Zhu, Z. Chen, L. Yu, S. Zeng, *Chirality*, 2012, **24**, 526-531.

30 G. Torkildsen, J. W. Proksch, A. Shapiro, S. K. Lynch, T. L. Comstock, *Clin. Ophthalmol.*, 2010, **4**, 331-341.

31 A. M. Badawy, A. K. Attiaa, A. E. Abd-Elaleem, M. M. Abd-Elmoety, S. G. Abd-Elhamid, *Int. J. Pharm. Anal.*, 2015, **40**, 1254-1268.

32 S. Kumar, M. Kumar, D. K. Majumdar, P. K. Sharma, C. L. Singh, A. Singh, *Indian J. Pharm. Sci.*, 2015, **77**, 399-404.

33 H. M. Ahmed, M. A. Mohamed, W. M. Salem, *Anal. Methods*, 2015, **7**, 581-589.

34 S. Akella, C. K. Mitra, *Indian J BiochemBiophys.* 2007, **44**, 82-87.

35 Y. Gao, T. Sun, C. Yang, B. Zhou, *Int. J. Electrochem. Sci.*, 2015**, 10**, 3230-3235.

36 R. A. Farghali, R. A. Ahmed, *Int. J. Electrochem. Sci.*, 2015, **10**, 1494-1505.

37 N. F. Atta, A. Galal, A. A. Wassel, A. H. Ibrahim, *Int. J. Electrochem. Sci.*, 2012, **7**, 10501-10518.

38 A. K. Attia, M. M. Abd-Elmoety, A. M. Badawy, A. E. Abd-Elaleemand, S. G. Abd-Elhamid, *Anal. Bioanal. Chem. Res.*, 2014, **1**, 138- 128.

39 N. F. Atta, A. Galal, S. M. Azab, *Int. J. Electrochem. Sci.* 2011, **6**, 5082-5096.

40 P. H. Rieger, *Electrochemistry*, Prentice-Hall International, New Jersey, 1987.

41 J. F. Rusling, *J. Colloids Surf.*, 1997, **123**, 81-88.

42 N. F. Atta, A. Galal, F. M. Abu-Attia, S. M. Azab, *Electrochim. Acta*, 2011, **56**, 2510–2517.

43 D. K. Gosser, *Cyclic Voltammetry: Simulation and Analysis of Reaction Mechanism*, VCH, New York, 1993.

44 E. Laviron, *J. Electroanal. Chem. Interfacial Electrochem.* 1979, **101**, 19-28.

45 A. J. Bard, L. R. Faulkner, *Electrochemical Methods: Fundamentals and Applications*, 2nd ed., Wiley, New York, 2001.

46 M. Y. Lachapelle, G. Drouin, Genetica. 2010, **139**, 199-207.

47 J. Premkumar, S. B. Khoo, *J. Electroanal. Chem.*, 2005, **576**, 105-112.

48 C. R. Raj, F. Kitamura, T. Ohsaka, *Analyst*, 2002, **9**, 1155-1158.

49 C. M. Anthony, M. D. Osselton, B. Widdop, *Clark's Analysis of Drugs and Poisons*, 3rd ed., Pharmaceutical Press, London, 2004.

50 J. Li, X. Q. Lin, *Anal. Chim. Acta*, 2007, **596**, 222-230.

51 S. A. John, *J. Electroanal. Chem.*, 2005, **579**, 249-256.

52 ICH Trapartite Guideline, validation of analytical procedures:Text and methodology, 2005, Q2(R1), 1-13. http://www.ich.org/cache/compo/276-254-1.html (Accessed:November 12, 2011).

Table 1 Regression data of linear range for quantitative determination of SPAR in human urine sample and BESI in Plasma samples.

Parameters	SPAR	SPAR in urine	BESI	BESI in plasma
Linearity range (mol L^{-1})	1.1×10^{-7}- 3.3×10^{-6}	2.22×10^{-7} - 2.80×10^{-6}	2.2×10^{-6}- 5.5×10^{-5}	8.0×10^{-6} - 4.5×10^{-5}
Slope	0.385	0.410	0.227	0.250
Intercept	8.56	7.88	7.85	6.85
Determination coefficient (r^2)	0.9976	0.9964	0.9984	0.9980
LOD (mol L^{-1})	2.87×10^{-8}	4.15×10^{-8}	3.76×10^{-7}	9.12×10^{-7}
LOQ (mol L^{-1})	9.57×10^{-8}	1.38×10^{-7}	1.25×10^{-6}	3.04×10^{-6}

Table 2 Comparison of the mentioned reported methods for the determination of SPAR and BESI.

Method	SPAR Linear range	Ref	BESI Linear range	Ref
Proposed Voltammetry (mol L^{-1}) (μg mL^{-1})	1.1×10^{-7} - 3.3×10^{-6} (0.047 - 1.415)	----	2.2×10^{-6}- 5.5×10^{-5} (0.947 - 23.67)	----
Voltammetry (mol L^{-1})	1×10^{-5} - 1×10^{-4} 2×10^{-7} - 1.4×10^{-6} 5×10^{-6} - 1.5×10^{-5} 2×10^{-7} - 6×10^{-5}	5 6 7 9	-------	----
Chromatography (μg mL^{-1})	3.0 - 14 5.0 - 80 25.0 - 150 30 - 90 1.0 - 80 1.0 -10 0.1 - 1.4 5.0 - 25	8 9 10 11 12 14 15 16	20 - 80	25
Spectrophotometry (μg mL^{-1})	0.02 - 0.20 5.0 - 25 10 - 60 5.0 - 25 0.5 - 7.0 20 - 100	17 18 19 20 21 22	2.5 - 80 3.0 - 30	29 30

Table 3 Recovery data obtained by standard addition method for SPAR and BESI in pharmaceutical formulations.

Drug	Spara tablets				Besivance eye drops			
	10^{-7} mol L^{-1}			%Recovery	10^{-6} mol L^{-1}			%Recovery
	Added	Taken	Found		Added	Taken	Found	
	2.00		6.020	100.3	2.00		6.030	100.50
	6.00		10.01	100.1	6.00		10.03	100.30
	8.00	4.0	12.03	100.2	8.00	4.0	12.01	100.08
	10.0		13.99	99.92	10.0		13.98	99.850
	12.0		16.04	100.2	12.0		16.02	100.10
[a]Mean				100.14				100.17
SD				0.143				0.2350
[b]RSD				0.142				0.2340

[a]Mean for five determinations

[b]RSD = (SD/ mean) x 100

SD is the standard deviation

Table 4 Evaluation of the accuracy and precision of the proposed method for the determination of SPAR in urine sample and BESI in plasma sample.

Drug	SPAR			BESI		
	10^{-7} mol L^{-1}		%Recovery	10^{-6} mol L^{-1}		%Recovery
	Added	Found		Added	Found	
	2.00	2.020	101.00	2.00	2.040	102.0
	6.00	5.990	99.800	6.00	6.010	100.1
	8.00	8.030	100.30	8.00	8.020	100.2
	10.0	10.04	100.40	10.0	10.02	100.2
	12.0	12.01	100.08	12.0	12.04	100.3
[a]mean			100.30			100.5
SD			0.4460			0.808
[b]RSD			0.4447			0.804

[a]Mean for five determinations

[b]RSD = (SD/ mean) x 100

Figure captions:

Fig. 1 Cyclic voltammograms of 1 x 10^{-3} mol L^{-1} SPAR at CPE in BR buffers of pH values ranging from 2 to 9 at scan rate of 100 mVs^{-1}, the linear relations of peak current and potential as a function of pH.

Fig. 2 Cyclic voltammograms of 1 x 10^{-3} mol L^{-1} BESI at CPE in BR buffers of pH values ranging from 2 to 9 at scan rate of 100 mVs^{-1}, the linear relations of peak current and potential as a function of pH.

Fig. 3 The linear relations of peak current of 1 x 10^{-3} mol L^{-1} SPAR and BESI at CPE in BR buffers of pH 2 at scan rate of 100 mVs^{-1}as a function of surfactants types.

Fig. 4 Cyclic voltammograms of 1 x 10^{-3} mol L^{-1} SPAR and BESI in BR buffer of pH 2 at scan rate of 100 mV s^{-1} recorded at four different working electrodes: CPE, CPE/SDS, AuCPE and AuCPE/SDS.

Fig. 5 Cyclic voltammograms of 1 x 10^{-3} mol L^{-1} SPAR and BESI solutions at AuCPE/SDS in BR buffer of pH 2 at different scan rates varied from 25 to 250 mV s^{-1}. The insets: Plots of the relation between logarithm of the oxidation peak currents of SPAR and BESI and the logarithm of the scan rates.

Fig. 6 The proposed electro-oxidation mechanisms of SPAR and BESI.

Fig. 7 Effect of accumulation time of 1 x 10^{-3} mol L^{-1} SPAR and BESI solutions at AuCPE/SDS in BR buffer of pH 2 at scan rate of 100 mV s^{-1}.

Fig. 8 Chronoamperograms for SPAR at AuCPE/SDS in BR buffer of pH 2.0 (A). Insets: I vs. $t^{1/2}$ from Cottrell's plot obtained from chronoamperograms (B) and the plot of the slopes of the straight lines against SPAR concentrations (C). Plots of response time (D) and recovery time (E) against SPAR concentrations.

Fig 9 Chronoamperograms for BESI at AuCPE/SDS in BR buffer of pH 2.0 (A). Insets: I vs. $t^{1/2}$ from Cottrell's plot obtained from chronoamperograms (B) and the plot of the

slopes of the straight lines against BESI concentrations (C). Plots of response time (D) and recovery time (E) against BESI concentrations.

Fig. 10 The differential pulse voltammograms of SPAR and BESI in presence of AA and UA mixture in BR buffer of pH 2 at AuCPE/SDS at scan rate of 10 mV s^{-1}.

Fig. 11 The effect of changing the concentration of SPAR and BESI at AuCPE/SDS in BR buffer pH 2 and scan rate 10 mV s^{-1}. The insets: The calibration plots of the oxidation peak current versus the concentration range of SPAR and BESI.

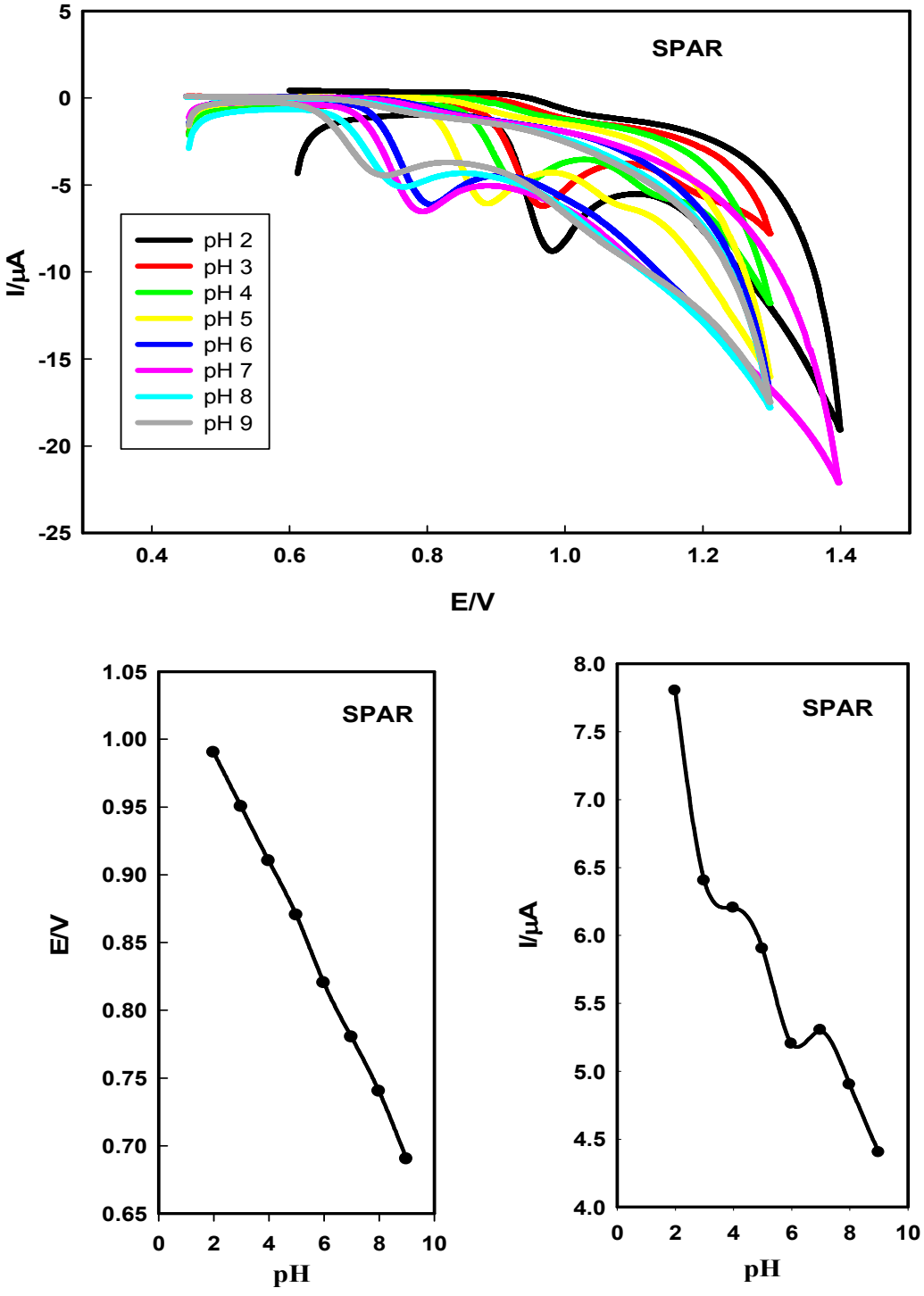


Figure 1

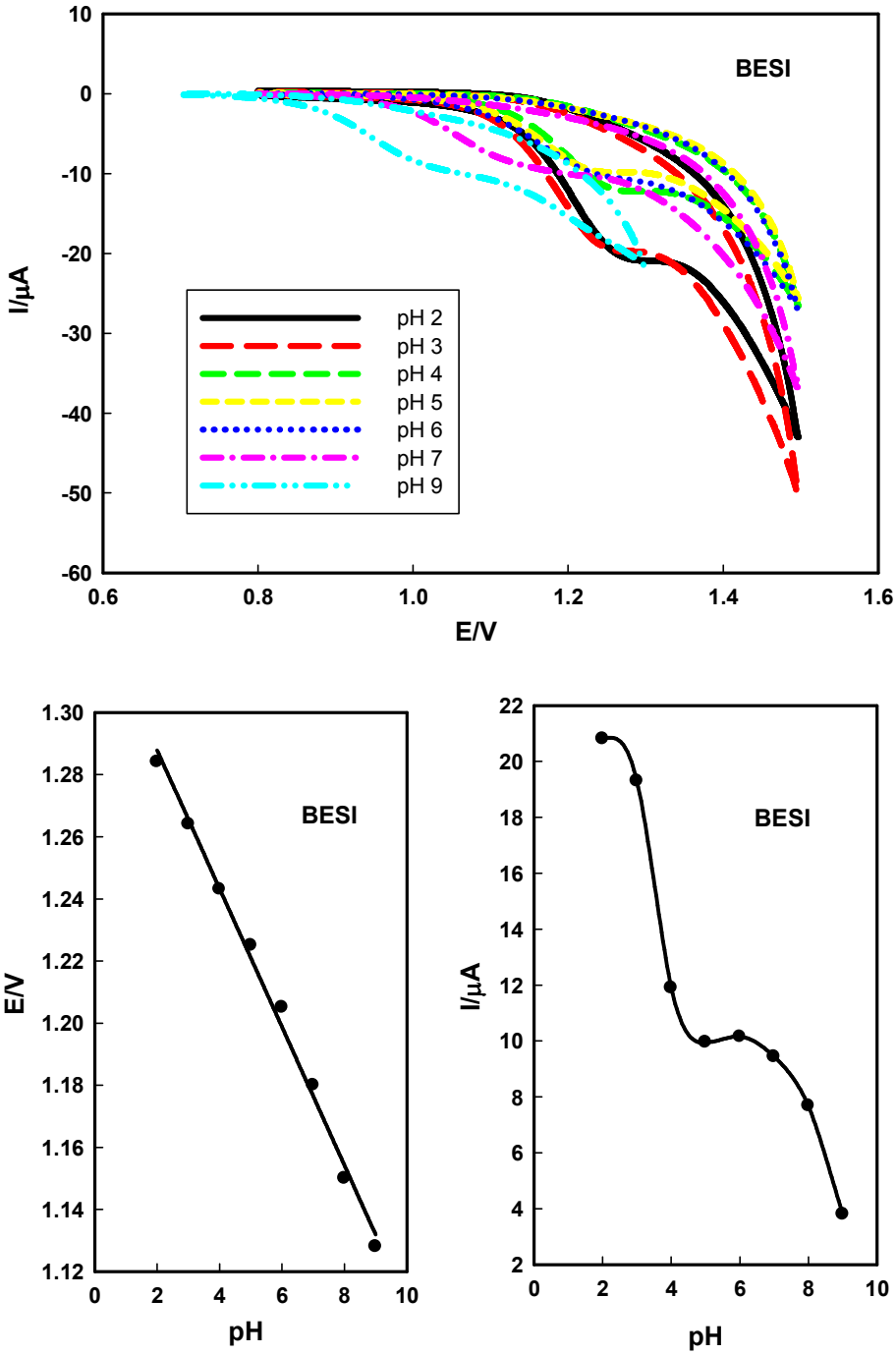
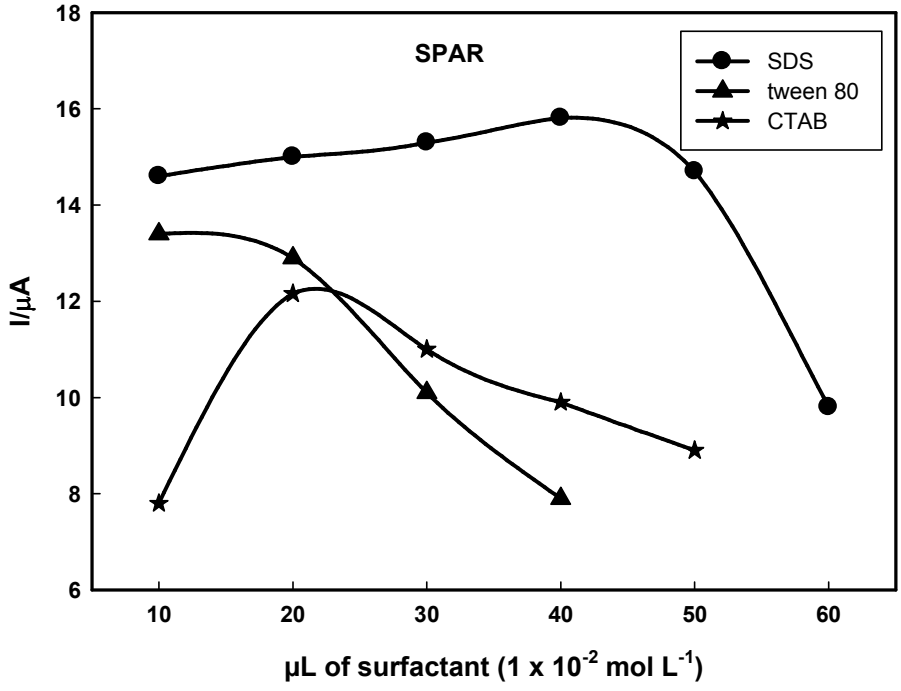


Figure 2



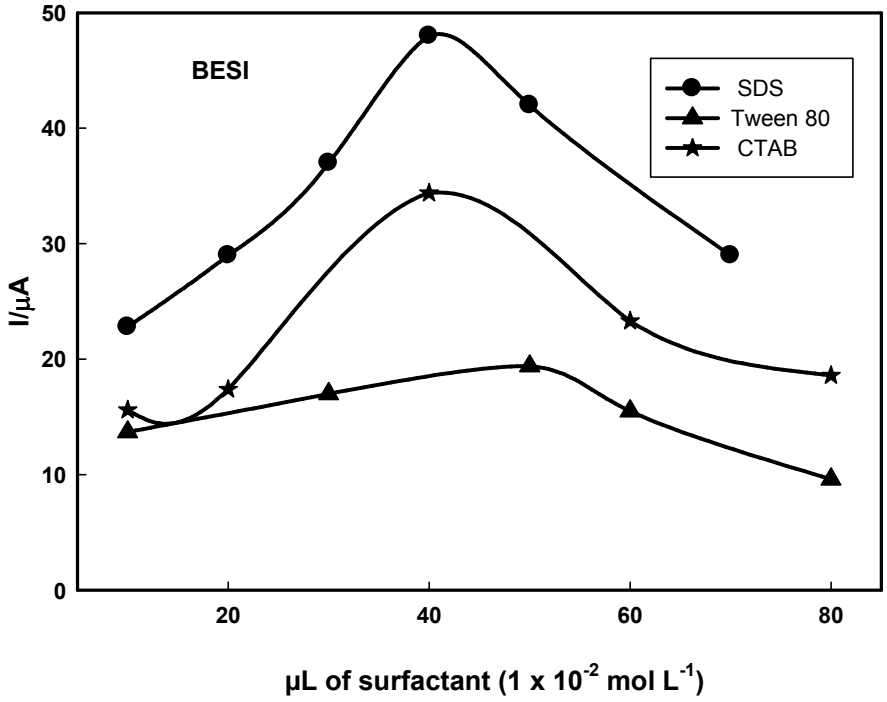
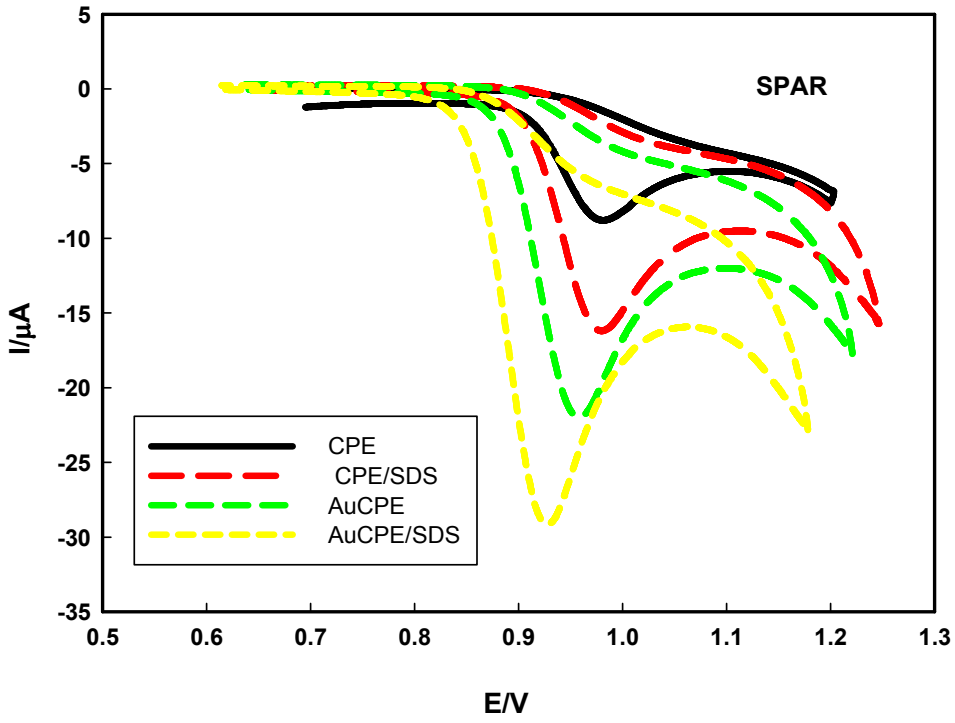


Figure 3



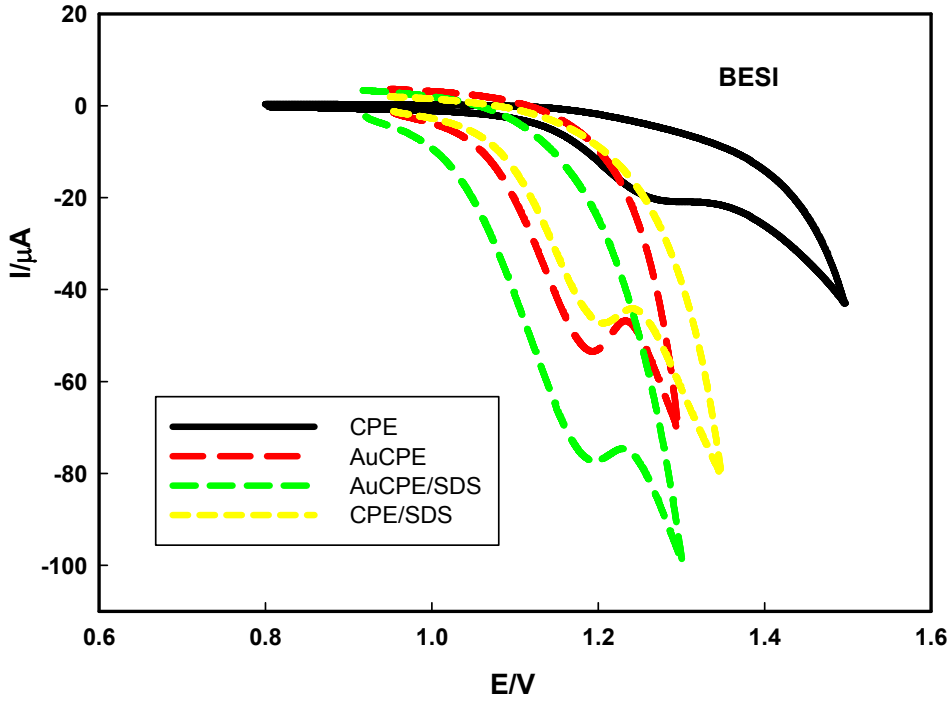


Figure 4

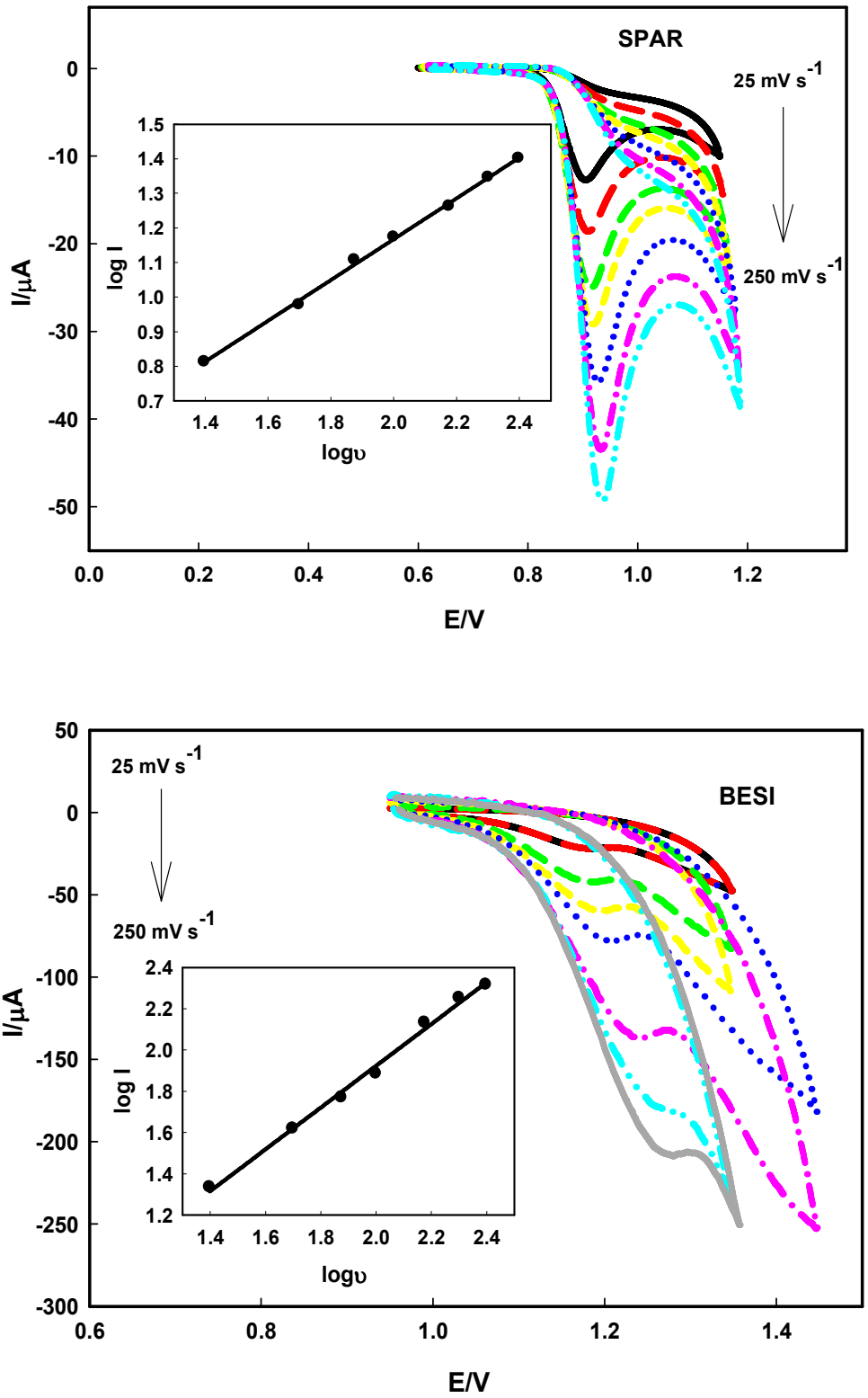


Figure 5

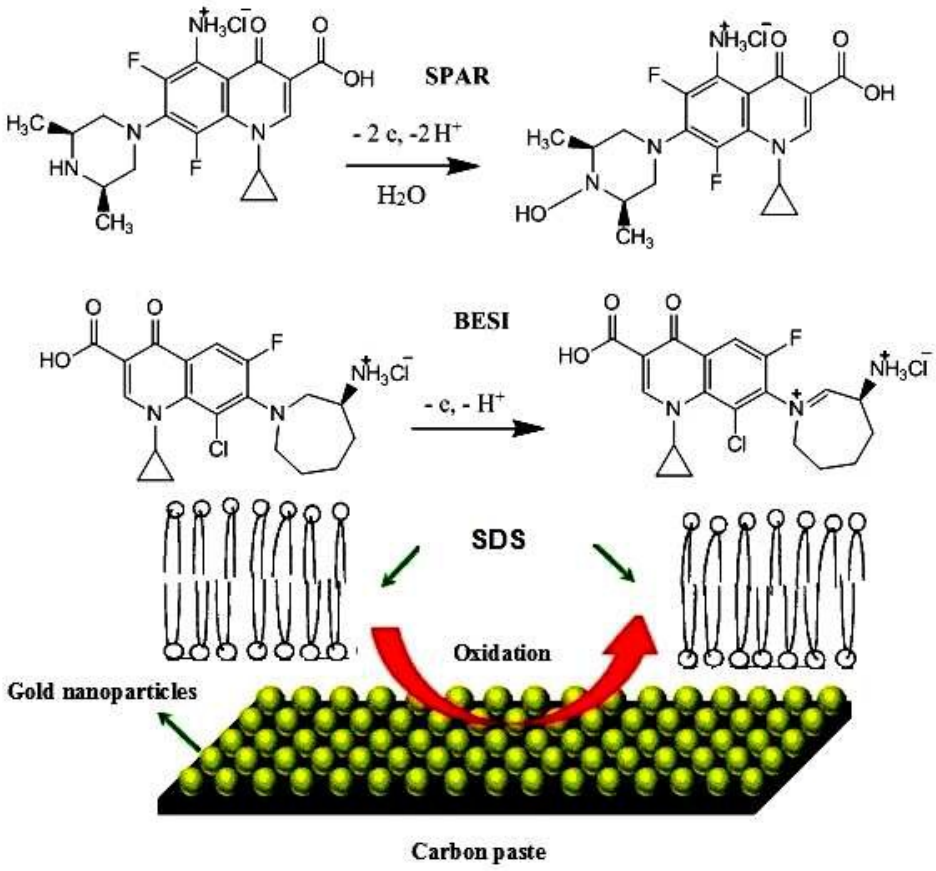


Figure 6

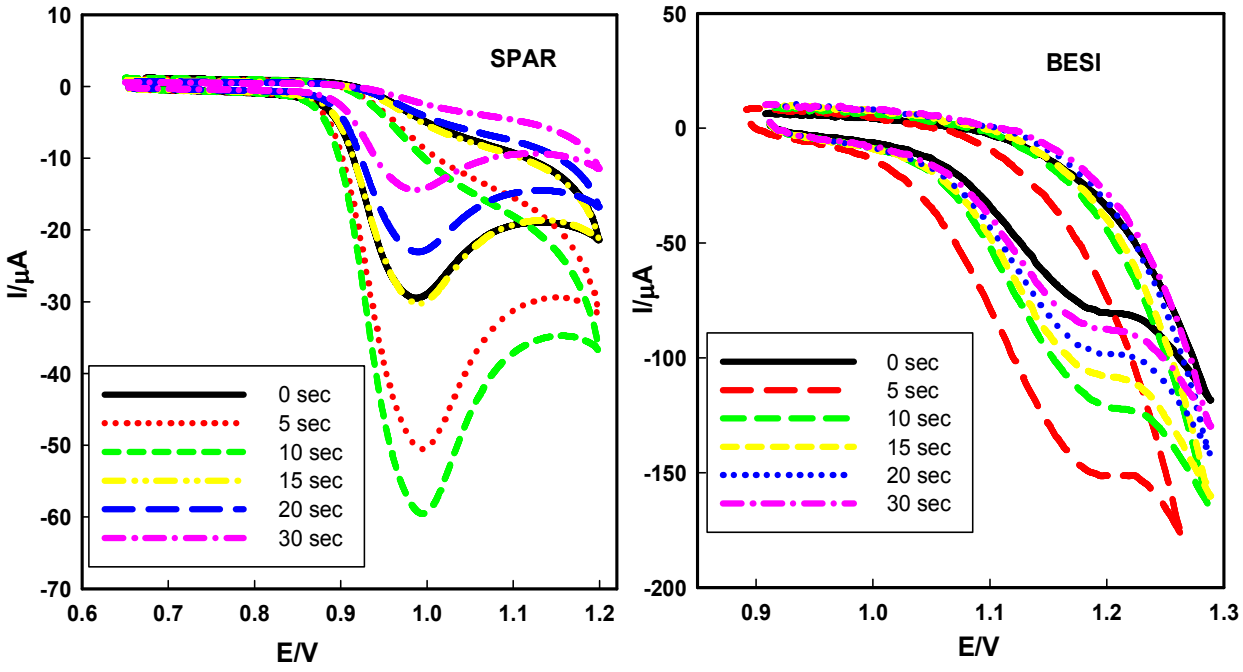


Figure 7

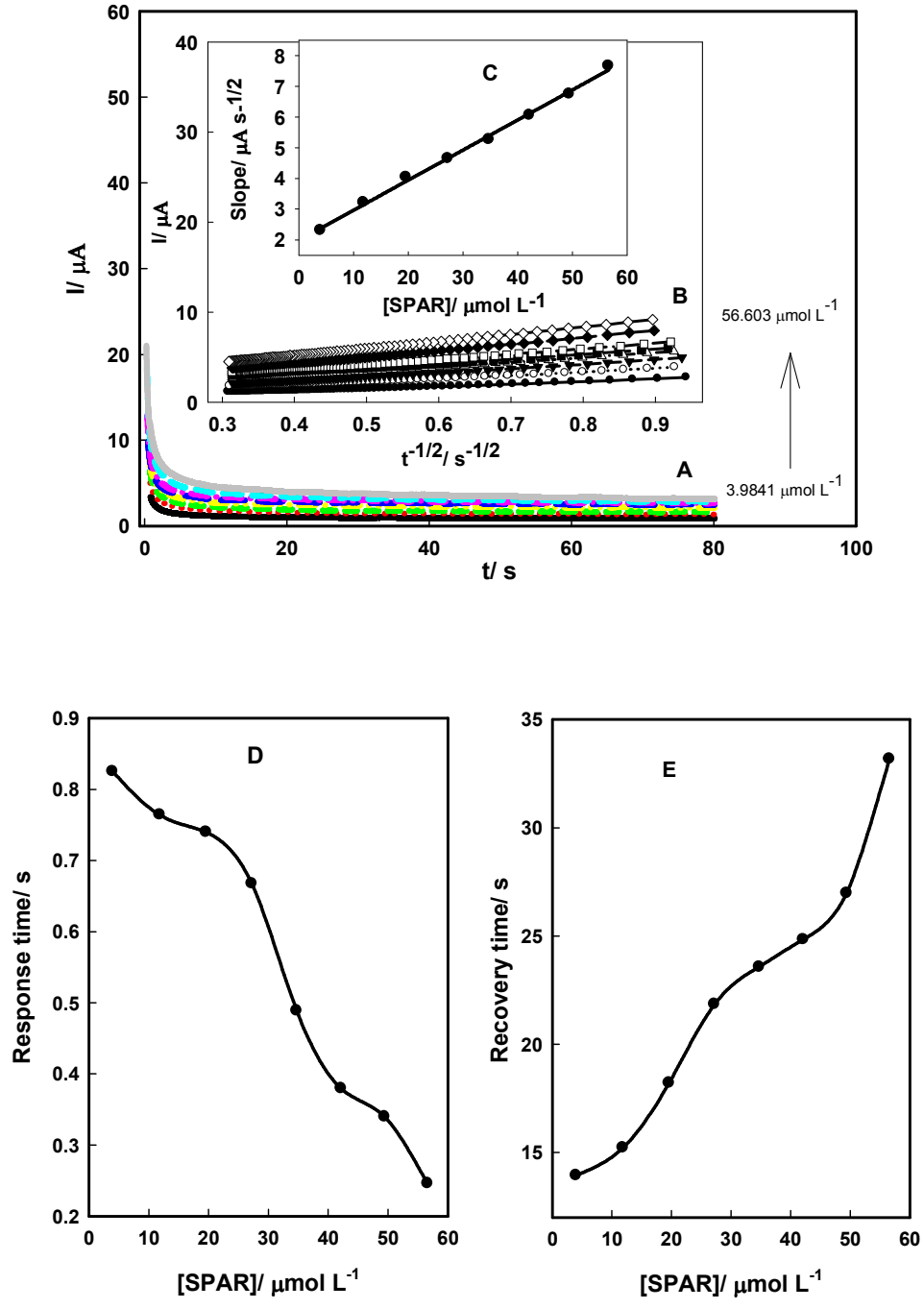


Figure 8

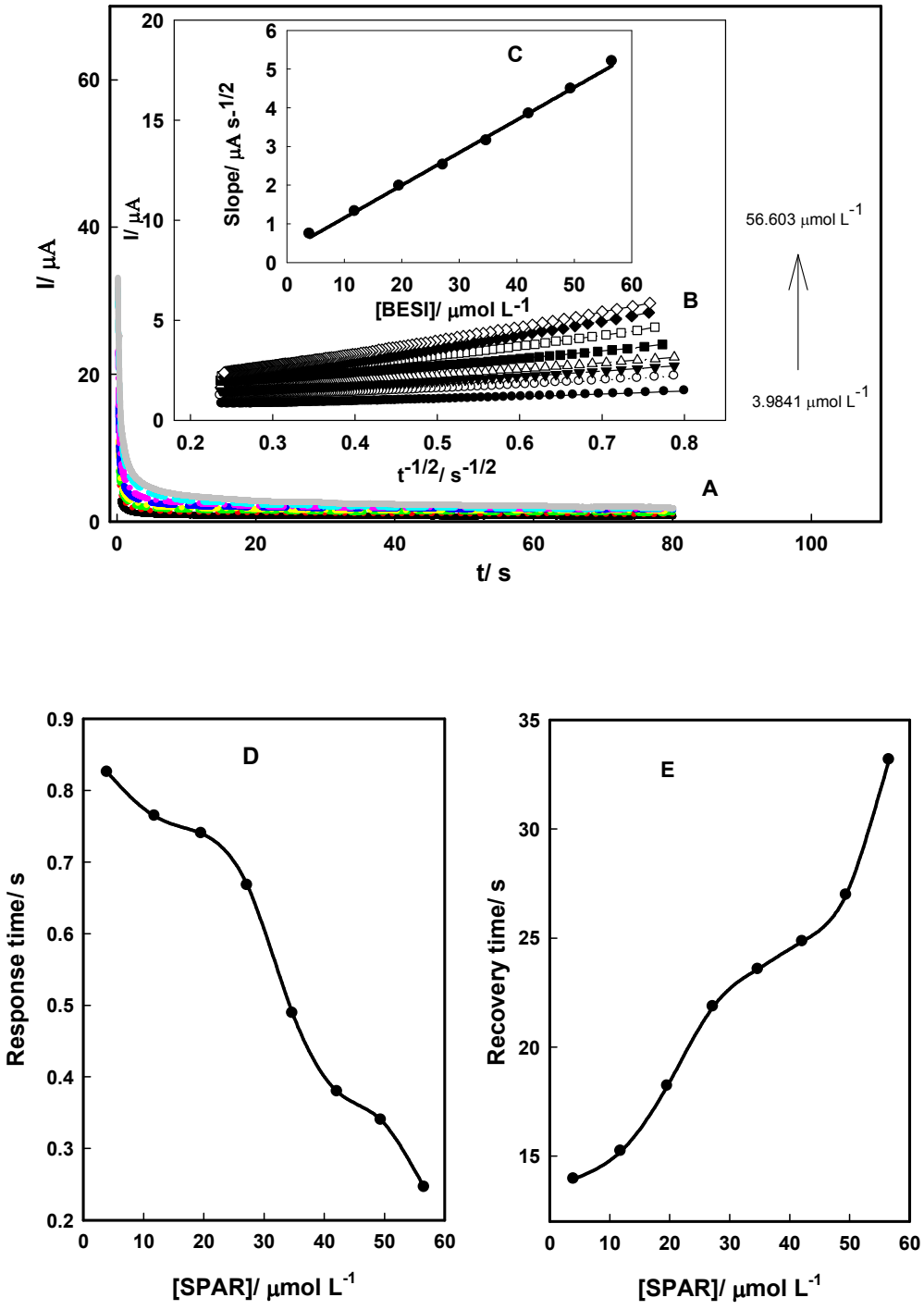


Figure 9

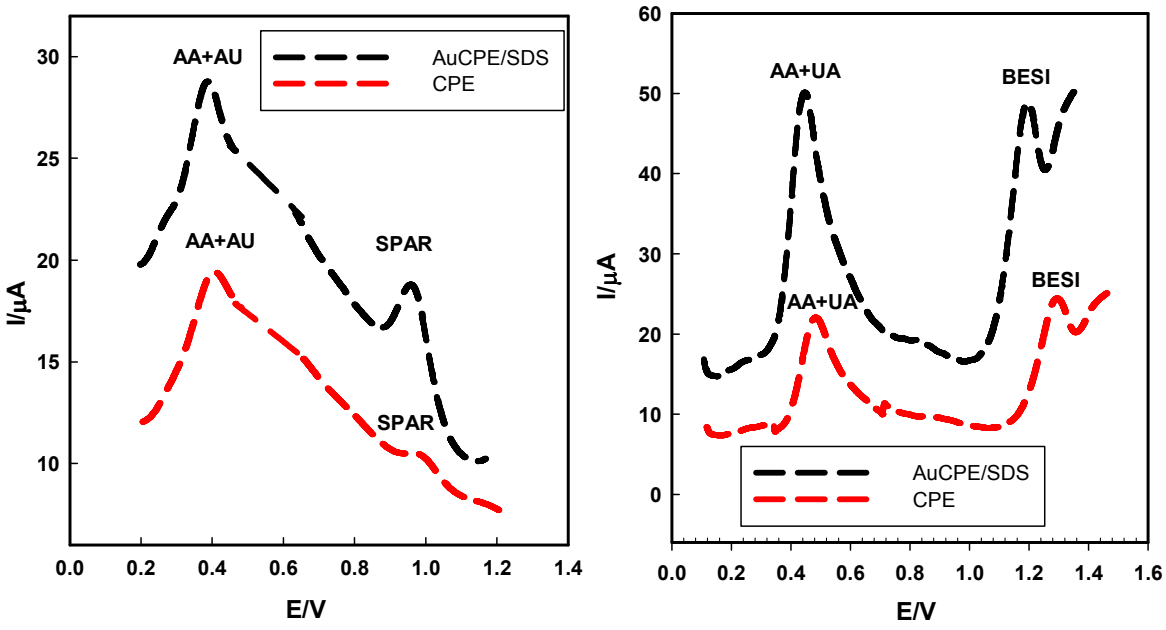


Figure 10

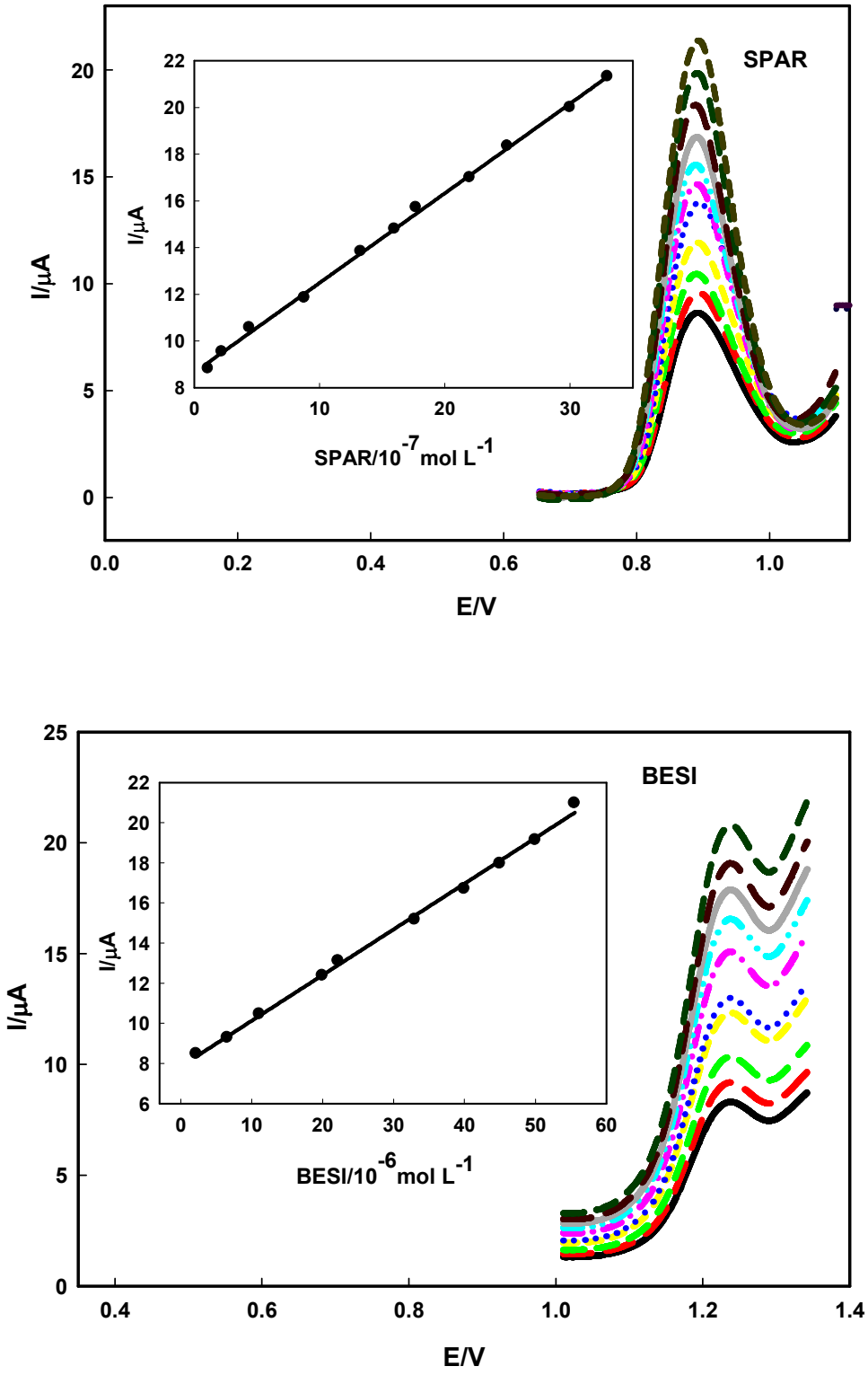
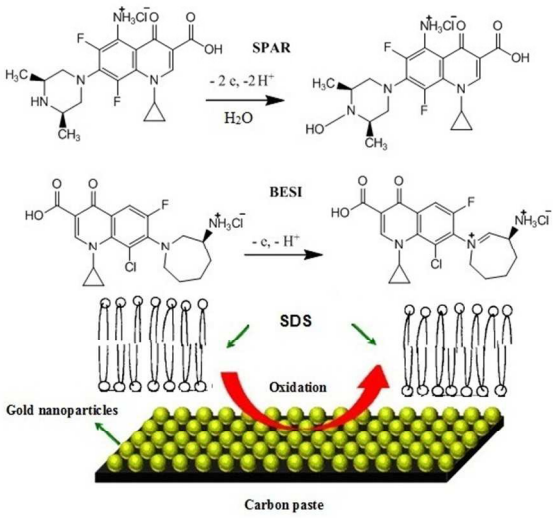


Figure 11



269x147mm (96 x 96 DPI)